

Research article

Potential host shift of the small hive beetle (*Aethina tumida*) to bumblebee colonies (*Bombus impatiens*)

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Abstract. Here we explored the potential for host shift from honeybee, *Apis mellifera*, colonies to bumblebee, *Bombus impatiens*, colonies by the small hive beetle, a nest parasite/scavenger native to sub-Saharan Africa. We investigated small hive beetle host choice, bumblebee colony defence as well as individual defensive behaviour of honeybee and bumblebee workers. Our findings show that in its new range in North America, bumblebees are potential alternate hosts for the small hive beetle. We found that small hive beetles do invade bumblebee colonies and readily oviposit there. Honeybee colonies are not preferred over bumblebee colonies. But even though bumblebees lack a co-evolutionary history with the small hive beetle, they are able to defend their colonies against this nest intruder by removal of beetle eggs and larvae and stinging of the latter. Hence, the observed behavioural mechanisms must be part of a generalistic defence system suitable for defence against multiple attackers. Nevertheless, there are quantitative (worker force) and qualitative differences (hygienic behaviour) between *A. mellifera* and *B. impatiens*.

Keywords: *Aethina tumida*, bumblebees, host shift, invasive species, parasites.

Introduction

As global travel and transportation of goods increases, biological invasions are happening more and more frequently (Mooney and Cleland, 2001; Levine and D'Antonio, 2003; Cassey et al., 2005). Introduced patho-

gens and parasites may switch hosts, thus posing new threats to native species. Due to their lack of co-evolutionary history, these new hosts do not possess any specific defence mechanisms against the new pest, having to rely entirely on generalistic means, which may or may not provide them sufficient resistance.

The small hive beetle, *Aethina tumida*, may be such an invasive parasite. It is native to sub-Saharan Africa, where it is a parasite and scavenger of honeybee, *Apis mellifera*, colonies (Lundie, 1940; Schmolke, 1974; Hepburn and Radloff, 1998; Neumann and Elzen, 2004). During the past decade the small hive beetle has been introduced into several countries around the world (Elzen et al., 1999; Mostafa and Williams, 2002; Animal Health Australia, 2003; Ritter, 2004; Clay, 2006). In North America and Australia the beetle has become well established (Evans et al., 2003; Hood, 2004; Neumann and Elzen, 2004; Spiewok et al., 2007), and its spread in these new ranges has been facilitated by the managed and feral populations of European honeybees. European honeybee subspecies, themselves not native to the New World and Australia (Goulson, 2003a; Moritz et al., 2005), appear to be more susceptible to small hive beetles than African ones, i.e. they suffer greater damage from beetle infestations and colonies collapse more often (Elzen et al., 1999, 2000), thus enhancing beetle reproduction. However, while honeybee colonies constitute a good resource for the small hive beetle, switching to alternate hosts would be a survival strategy where beehives are less abundant or temporarily unavailable (e.g. when hives have been moved by beekeepers).

Recent evidence suggests that the small hive beetle may be less host specific than previously thought. It has

been found to naturally infest commercial bumblebee colonies in the field in North America (*Bombus impatiens*, Spiewok and Neumann, 2006a). Moreover, the beetle can successfully reproduce in laboratory *B. impatiens* colonies (Stanghellini et al., 2000; Ambrose et al., 2000) as well as in managed hives of Australian stingless bees (R.B. Luttrell, pers. comm.).

Studies with honeybees demonstrated that a mixture of odour cues from bees and hive products are attractive to small hive beetles (Elzen et al., 1999, 2000; Suazo et al., 2003; Torto et al., 2005). Since hive products such as wax, honey, pollen and brood, along with bees, can also be found in bumblebee and stingless bee colonies (Dollin, 1996; Michener, 2000), they are not unlikely to attract the beetles. Indeed, Spiewok and Neumann (2006a) showed that small hive beetles are attracted by bumblebee workers and bumblebee-collected pollen. The similarities between honeybee and bumblebee colonies also allow other macroparasites of the Apidae, as for instance the greater wax moth, *Galleria mellonella*, and the bumblebee wax moth, *Aphomia sociella*, to switch between *Bombus* and *Apis* hosts (Williams, 1997). As studies on small hive beetle rearing revealed, the actual foodstuffs on which the adults and larvae feed do not have to be very specific, as long as they contain sufficient proteins for egg production and larval growth (Ellis et al., 2002).

A range of different behavioural defence strategies against the small hive beetle have been documented in honeybees: social encapsulation (Neumann et al., 2001a; Ellis et al., 2003a, 2004a), removal of beetle eggs (Ellis et al., 2003b, 2004b; Neumann and Härtel, 2004; Spiewok and Neumann, 2006b) and larvae (jettisoning behaviour: Lundie, 1940; Schmolke, 1974; Neumann and Härtel, 2004; Spiewok and Neumann, 2006b), aggression (Schmolke, 1974; Elzen et al., 2001) and absconding (Hepburn and Radloff, 1998; Hood, 2000). Since all these mechanisms have been observed not only in African honeybees, which share a co-evolutionary history with the small hive beetle, but also in European subspecies that only recently came into contact with this novel pest, they must be part of the suite of general defensive behaviours present in all honeybees (Michener, 1974; Thompson, 1994). Therefore, it appears that only quantitative differences in these behaviours account for the difference in the ability of African and European honeybees to deal with the beetles (Neumann and Elzen, 2004). Other social bee species may show similar defence mechanisms if confronted with small hive beetles, but to date this has not been studied.

Although it has been mentioned in the literature that the small hive beetle may pose a potential threat to the social insect fauna indigenous to North America (Tonn, 2002; Tonn et al., 2006), the actual attractiveness and vulnerability of bumblebees, which are not native to sub-Saharan Africa but occur in North America (Michener, 2000), has not been studied in depth. With the evidence presented by Ambrose et al. (2000), Stanghellini et al. (2000) and Spiewok and Neumann (2006a) suggesting

that the small hive beetle could exploit bumblebee colonies, we decided to further investigate the ability of small hive beetles to locate and infest bumblebee nests and the defence mechanisms that counteract such infestations. We conducted these studies to help evaluate the threat of small hive beetles to native pollinators and to contribute further knowledge to our understanding of the general defence mechanisms of social bees against nest intruders.

Materials and methods

All experiments were conducted in the summer of 2005 in Maryland, USA.

Experiment A: Transmission and host choice

Four queenright commercial *B. impatiens* colonies and four small (nucleus) queenright honeybee colonies of mixed European origin (predominantly *A. m. ligustica*) were set up in a greenhouse (Fig. 1). Honeybee mating nuclei were chosen to match the size of the bumblebee colonies, so as to provide a similar amount of odour cues for beetle orientation (Torto et al., 2005; Spiewok and Neumann, 2006a). All colonies were placed in new unused standard single ten-frame Langstroth hive boxes to give them a similar external appearance, with four empty boxes serving as controls. The boxes were divided into four groups, each consisting of one bumblebee colony, one honeybee colony and one control (Fig. 1). These were spaced evenly within the greenhouse. The distances between boxes within a group were 40 cm and between groups 143 cm. Distances to the beetle release box placed in the centre (Fig. 1) were 145 cm and 183 cm, respectively. For the duration of the experiment, the colony entrances were closed with wire mesh (3.2 openings per cm² of mesh) allowing passage of small hive beetles but preventing the bees from flying. Adult small hive beetles (N = 1,000) were released in the beetle release box at dusk (19:00), in the time window for natural flight activity of small hive beetles (Schmolke, 1974; Elzen et al., 2000), and given 36 hours to disperse. During the experiment, mean temperature in the greenhouse was 32°C. For cooling, all colonies and the controls were sprayed at noon with water using a manual pump sprayer. The experiment was terminated at dawn on the second day by putting all colonies in individual plastic bags and deep-freezing them at -80°C. All beetles found in the control boxes, in the beetle release box and outside of the boxes were collected. The colonies were then systematically dissected to assess colony phenotype, number of adult beetles, as well as the presence of small hive beetle eggs and larvae. The following phenotype data were evaluated: colony weight (nest structure, storage pots, brood cells, and bees), number of bees and number of brood cells.

Experiment B: Colony defence by removal of small hive beetle life stages

The removal of small hive beetle life stages was investigated in ten queenright commercial *B. impatiens* colonies following standard protocols used with African honeybees (Neumann and Härtel, 2004; Spiewok and Neumann, 2006b). Six colonies were set up in the field, and four colonies were placed in the laboratory. The latter were connected to individual entrance holes via plastic tubes (length 20 cm; Ø 4 cm), enabling the bees to fly and forage freely in the field.

1. Removal of eggs: To obtain small hive beetle eggs, 100 adult beetles were reared in the laboratory (Neumann et al., 2001b; Mürrle and Neumann, 2004) and introduced into ten plastic jars containing water, a protein diet made of honey and pollen (1:2), and two double microscope slides spaced by cover slips and held together with paper clips. Small hive beetle females readily oviposit between microscope slides,

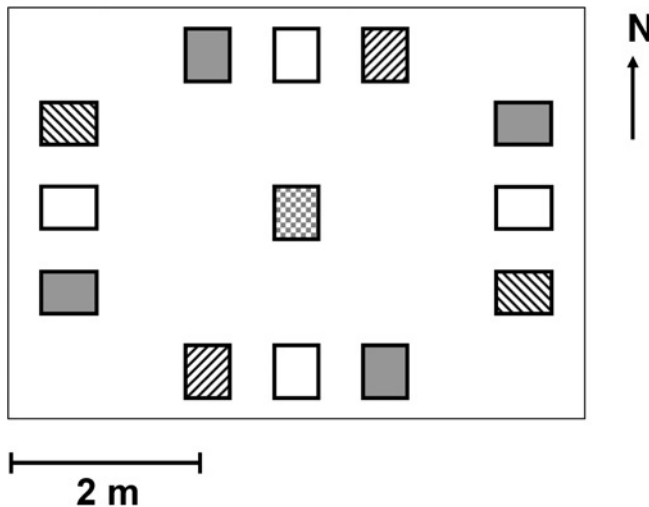


Figure 1. Setup for experiment A: transmission and host choice in the greenhouse (shaded boxes = honeybee colonies; striped boxes = bumblebee colonies, white boxes = controls, chequered box = beetle release box).

as they naturally prefer small cracks (Lundie, 1940) to protect their eggs from being removed by host bees (Neumann and Härtel, 2004). One double microscope slide with protected eggs and two single slides (a double slide opened up) with now exposed, unprotected eggs (~100 eggs/slide; total $N = 930$ protected eggs and 1249 unprotected eggs) were introduced into each of the ten colonies on top of the involucrum (cover made of wax and other materials, which is typically found over the inner nest of bumblebee colonies, Wilson, 1971). After one, three, five, ten and 24 hours the slides were briefly removed from the colonies and, before being reintroduced at the same within-box location, the remaining eggs on the slides were counted.

2. Removal of larvae: Small hive beetle larvae were reared in the laboratory (see above). Larvae not covered with sticky coating ("dry" rearing approach, Neumann and Härtel, 2004) were placed in open Petri dishes ($N = 50$ larvae each) and introduced into each of the test colonies on top of the involucrum. After one, two, four, seven, ten and 24 hours the Petri dishes were briefly removed from the colonies and the remaining larvae were counted before being reintroduced at the same location. Larvae that were found killed in the Petri dishes were considered removed, since they did not pose a threat to the colony any more. To control for the escape rate of larvae, three Petri dishes containing 50 larvae each were set up in a plastic box and kept in darkness in the laboratory. The number of remaining larvae in these control dishes was recorded at the respective time intervals.

3. Colony phenotypes: After the removal experiments, the colonies set up in the field were euthanized by deep-freezing at -80°C and colony phenotype data were evaluated as described above.

Experiment C: Individual behavioural defence mechanisms of workers

Four queenright commercial *B. impatiens* colonies set up in the laboratory were equipped with glass lids to facilitate observations conducted under red light conditions. One microscope slide with unprotected small hive beetle eggs (see above; $N = 100$ eggs/slide) was introduced into each of the four colonies on top of the involucrum. Bare microscope slides served as controls. The colonies were given $\frac{1}{2}$ hour to settle after the disturbance. The behaviour of workers on the slides was then observed for 20 minutes in each colony and recorded according to the following categories: 1) ignoring (walking, resting, self grooming or ventilating on slide), 2) investigating (antennating/licking slide surface

or eggs), 3) attacking (biting/eating of eggs, biting slide). Afterwards, the remaining eggs on the slides were counted.

Small hive beetle larvae not covered with sticky coating were reared in the laboratory as described above. An empty Petri dish was introduced into each test colony on top of the involucrum to serve as a behavioural arena that could be monitored. Given some time to settle after the disturbance, bumblebee workers treated the Petri dishes indifferently (no investigations, no stopping when passing by or running over the dish). After $\frac{1}{2}$ hour, a single larva was introduced into the arena through a hole in the lid, and behavioural interactions were observed for five minutes. Pieces of rubber band (length = 1 cm) resembling small hive beetle larvae in size and shape served as controls. The following information was recorded: 1) time to first worker entering the Petri dish, 2) time to first investigation (antennating/licking), 3) number of subsequent investigations, 4) time to first attack (grasping, biting or stinging), 5) number of subsequent attacks, 6) killing or removal. If larvae or controls were still remaining $\frac{1}{2}$ hour after introduction, they were removed from the Petri dishes. Then, colonies were given an additional $\frac{1}{2}$ hour to settle before the next larva was introduced. This was repeated six times for each colony for a total of 24 larvae and 24 controls.

These observations were also conducted in the same fashion in four three-frame honeybee observation hives that were set up in the laboratory (population ~3000 bees each). Here the eggs, larvae and controls, respectively, were introduced into the Perspex runways leading to the combs, which the bees used as the entryway to the hive.

Data analyses

Experiment A: Transmission and host choice

The numbers of workers in honeybee versus bumblebee colonies were compared using a Mann-Whitney U-test. The numbers of small hive beetles found in controls, honeybee and bumblebee colonies were analysed by a Kruskal-Wallis test and multiple comparisons as post hoc tests. Simple correlations (r-matrix) were performed between the number of workers in a colony or colony weight, respectively, and number of small hive beetles found in that colony.

Experiment B: Colony defence by removal of small hive beetle life stages

Mann-Whitney U-tests were performed to test for differences in removal rates of protected versus unprotected eggs, for differences between removal rates of larvae and controls and for differences in removal rates of eggs and larvae between laboratory and field colonies. Simple correlations (r-matrix) were performed between the colony phenotype data and the removal rates for small hive beetle life stages.

Experiment C: Individual behavioural defence mechanisms of workers

Mann-Whitney U-tests were performed to test for differences in removal rates and number of investigations of eggs, larvae and controls between bumblebees and honeybees, and in removal rates and number of investigations between treatments and controls. For the behavioural categories ignoring, investigating and attacking of eggs, behavioural ratios were calculated for each replicate of the 20 min. observation period by dividing the number of observations of each specific behaviour by the mean number of all behaviours observed. These behavioural ratios were compared between honeybees and bumblebees and between treatments and controls using Mann-Whitney U-tests. Before comparing time to first investigation and time to first attack, respectively, between honeybees and bumblebees and between treatments and controls with Mann-Whitney U-tests, inevitable methodological differences were accounted for by subtracting from these values the time to first worker entering the Petri dish in the bumblebee colonies. All analyses were performed using the programmes SPSS® and Statistica®.

Results

Experiment A: Transmission and host choice

Mean number of workers did not differ significantly between honeybee and bumblebee colonies (honeybees 208 ± 71.41 [mean \pm SD], bumblebees 280.25 ± 86.55 ; $Z = -1.155$, $p > 0.05$). Upon termination of the experiment, no small hive beetles were found in the empty control boxes. We found a total of 121 small hive beetles in the honeybee nucleus colonies, and 292 in the bumblebee colonies (medians with [1. quartile; 3. quartile]: honeybees 18 [7; 42], bumblebees 70 [59; 84]; Fig. 2). Neither the number of workers ($r = 0.457$, $p > 0.05$) nor colony weight ($r = 0.700$, $p > 0.05$) were significantly correlated with the number of beetles found in the colonies. We found significant differences in the number of small hive beetles between controls, honeybee and bumblebee colonies ($H(2, N = 12) = 8.649$, $p < 0.02$). Significantly more small hive beetles were present in the bumblebee colonies than in the control boxes ($p < 0.02$), but no significant differences were found between beetle numbers in honeybee and bumblebee colonies or between honeybee colonies and controls ($p > 0.05$). In all four bumblebee colonies we found several small hive beetle egg clutches on brood cells (Fig. 3) and in storage pots. In one honeybee nucleus colony small hive beetle eggs were found in the debris.

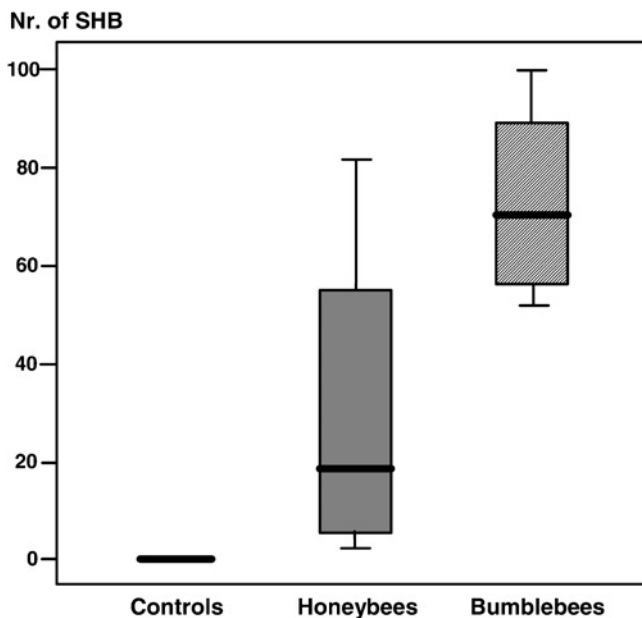


Figure 2. Experiment A: transmission and host choice. Number of small hive beetles (boxplot showing medians and quartiles) found in the control boxes, honeybee and bumblebee colonies ($N = 4$ each) 36 hours after release.

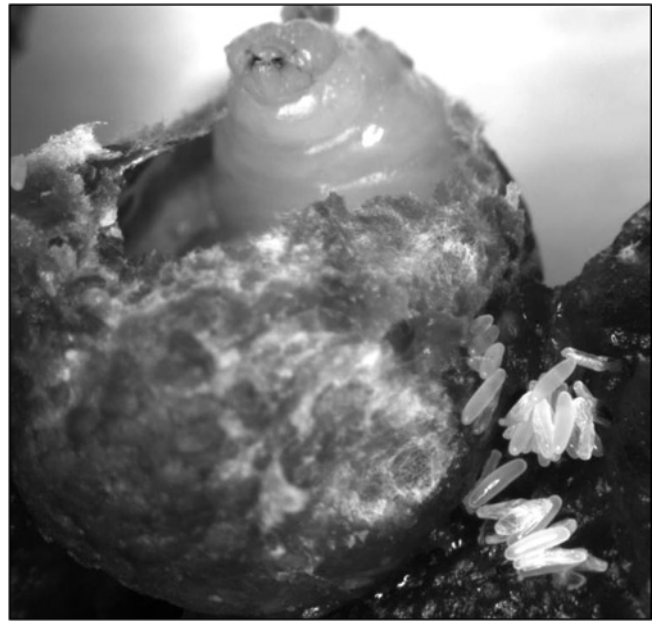


Figure 3. Small hive beetle egg clutch on brood cell in a bumblebee colony.

Experiment B: Colony defence by removal of small hive beetle life stages

1. Removal of eggs: Within 24 hours, 98.14 ± 3.25 % [mean \pm SD] of the unprotected eggs and 12.11 ± 8.80 % of the protected eggs were removed from the bumblebee colonies (Fig. 4). Significantly more protected eggs remained in the colonies as compared to unprotected eggs (after one hour: $Z = -3.785$, $p < 0.001$; after 24 hours: $Z = -3.862$, $p < 0.001$, Fig. 4).

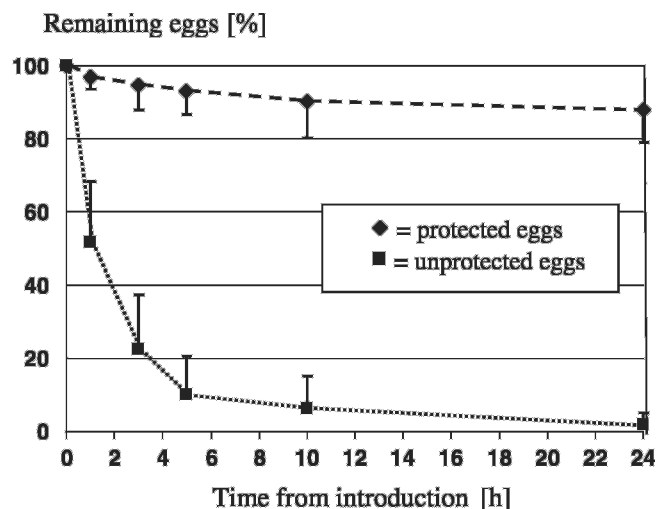


Figure 4. Experiment B: Removal of small hive beetle eggs (means \pm SD) after one, three, five, ten and 24 hours in ten *B. impatiens* colonies (diamonds = protected eggs, squares = un-protected eggs).

2. Removal of larvae: In the controls, 100 % of the larvae remained after 24 hours, whereas in the test colonies a significant proportion of larvae was removed (after one hour: $Z = -2.090$, $p < 0.04$; after 24 hours: $Z = -2.912$, $p < 0.005$, Fig. 5). In eight out of ten colonies all larvae were removed after 24 hours. Some of the larvae were stung by the bumblebee workers (Fig. 6a), but were not immediately removed. These larvae could be clearly distinguished by their purplish colour (Fig. 6b) and were dead. At least 22 stung larvae were found in nine colonies.

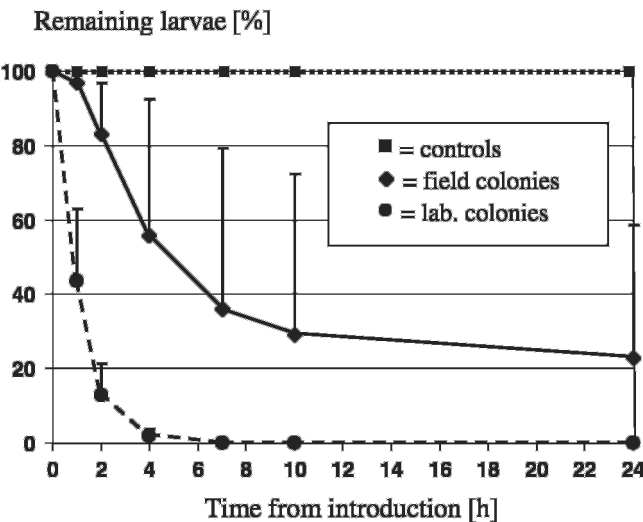


Figure 5. Experiment B: Removal of small hive beetle larvae (means \pm SD) after one, two, four, seven, ten and 24 hours in ten *B. impatiens* colonies (diamonds = field colonies, circles = laboratory colonies, squares = controls).

3. Differences in removal rates between laboratory and field colonies: No significant differences were found in the removal rates of protected or unprotected eggs between laboratory and field colonies ($Z = -1.279$, $p > 0.05$ for protected and $Z = -1.574$, $p > 0.05$ for unprotected eggs). Removal rates of larvae were significantly higher in the laboratory colonies during the first seven hours (after one hour: $Z = -2.574$, $p = 0.01$; after seven hours: $Z = -2.274$, $p < 0.03$), but later no significant differences could be found (after 24 hours: $Z = -1.217$, $p > 0.2$).

4. Colony phenotypes: Colony phenotype data for the *B. impatiens* field colonies are shown in Table 1, and the correlation r-matrix for colony phenotypes and removal of small hive beetle eggs and larvae in Table 2. After Bonferroni adjustment, none of the correlations was significant.

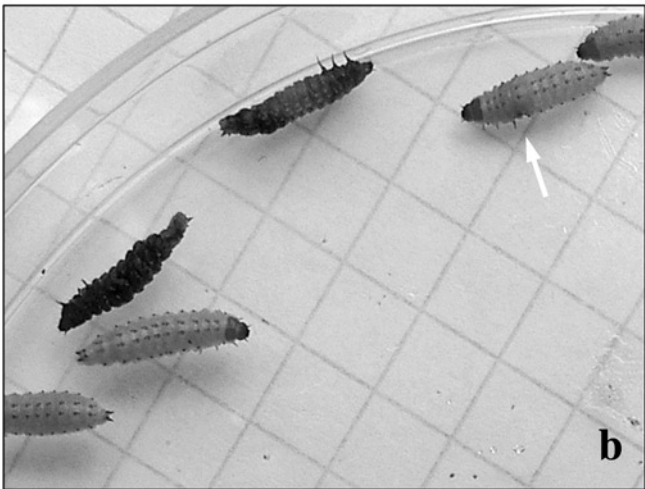


Figure 6. a) Bumblebee worker grasping a small hive beetle larva and attempting to sting. b) Stung small hive beetle larvae in various stages of discolouration. Arrow: fresh sting mark.

Table 1. Experiment B: Colony defence by removal of small hive beetle life stages. Colony phenotype data for the tested *B. impatiens* field colonies. Colony weight (nest structure, storage pots, brood cells, bees), number of workers and of brood cells are shown.

Colony	Colony weight [g]	# Workers	# Brood cells
1	110.4	69	19
2	164.9	164	111
3	162.5	119	170
4	182.9	191	176
5	149.2	146	140
6	155.2	105	148
Mean \pm SD	154.2 \pm 24.3	132.3 \pm 43.7	127.3 \pm 57.9

Table 2. Experiment B: Colony defence by removal of small hive beetle life stages. Correlation r-matrix for colony phenotype data and removal data for the tested *B. impatiens* field colonies. Colony weight, number of workers and of brood cells, removal of small hive beetle protected and unprotected eggs after three and 24 hours, and removal of small hive beetle larvae after two and 24 hours were considered. After Bonferroni adjustment (level of significance: $\alpha = 0.0021$), none of the correlations is significant. Asterisks indicate $r > 0.8$.

			Colony weight	# Workers	# Brood cells
Colony weight			1		
# Workers			0.864*	1	
# Brood cells			0.892*	0.650	1
Egg removal	Protected	3 hours	0.251	0.090	0.577
		24 hours	-0.226	0.023	-0.161
	Unprotected	3 hours	0.247	0.237	0.009
		24 hours	-0.354	-0.219	-0.639
Larva removal		2 hours	-0.062	0.129	-0.438
		24 hours	-0.564	-0.316	-0.859*

Table 3. Experiment C: Individual behavioural defence mechanisms of workers. Behavioural ratios [means \pm SD] of bumblebees and honeybees towards small hive beetle eggs for the 20 minutes observation period are shown.

	Ignore		Investigate		Attack	
	Eggs	Controls	Eggs	Controls	Eggs	Controls
Bumblebees	0.31 \pm 0.04	0.50 \pm 0.08	0.62 \pm 0.03	0.50 \pm 0.08	0.07 \pm 0.01	0.00 \pm 0.00
Honeybees	0.14 \pm 0.12	0.12 \pm 0.04	0.86 \pm 0.12	0.87 \pm 0.07	0.01 \pm 0.01	0.01 \pm 0.02

Experiment C: Individual behavioural defence mechanisms of workers

Within 50 minutes after introduction of unprotected small hive beetle eggs, 61.88 ± 8.25 % [mean \pm SD] were removed by the bumblebee workers, whereas the honeybees removed only 5.85 ± 8.54 % of eggs within the same time window. Thus, the bumblebees performed egg removal significantly faster ($Z = -2.323$, $p = 0.02$). Mean behavioural ratios for bumblebees and honeybees are shown in Table 3. In bumblebees, behavioural ratios of ignoring were significantly higher towards the controls ($Z = -2.309$, $p < 0.03$), whereas behavioural ratios of attacking were higher towards small hive beetle eggs ($Z = -2.366$, $p < 0.02$). Differences in behavioural ratios of investigating in bumblebees ($Z = -1.732$, $p > 0.05$) as well as in all behavioural ratios in honeybees between eggs and controls were not significant ($Z = 0.000$, $p = 1$ for ignoring and investigating, $Z = -0.331$, $p > 0.5$ for attacking). In bumblebees, behavioural ratios of ignoring were significantly higher ($Z = -2.021$, $p < 0.05$ for eggs and $Z = -2.309$, $p < 0.03$ for controls), and of investigating significantly lower ($Z = -2.309$, $p < 0.03$ for eggs and controls) as compared to honeybees. Behavioural ratios of attacking were significantly higher in bumblebees towards eggs ($Z = -2.323$, $p = 0.02$), but did not differ significantly towards controls ($Z = -0.189$, $p > 0.5$).

Within 30 minutes after introduction, the bumblebees killed or removed 41.67 ± 28.87 % of small hive beetle larvae (Fig. 6) and removed none of the controls. The honeybees killed or removed 62.50 ± 15.96 % of larvae

and removed 16.67 ± 13.61 % of controls. The difference between removal rates of larvae in honeybees versus bumblebees was not significant ($Z = -1.323$, $p > 0.05$), but significantly more controls were removed by the honeybees as compared to the bumblebees ($Z = -2.000$, $p < 0.05$). In bumblebees, no significant difference was found in the number of investigations between larvae and controls ($Z = -1.732$, $p > 0.05$), whereas in honeybees the controls were significantly more often investigated than the larvae ($Z = -2.309$, $p < 0.03$). Honeybees started to investigate the larvae significantly earlier (29 ± 64 seconds after introduction) than bumblebees (after 90 ± 82 seconds; $Z = -3.981$, $p < 0.001$), but time to first investigation of the controls did not differ significantly (honeybees: 26 ± 43 seconds, bumblebees: 31 ± 32 seconds; $Z = -1.827$, $p > 0.05$). In bumblebees, time to investigation of the controls was significantly shorter as compared to treatments ($Z = -3.017$, $p < 0.005$), whereas in honeybees the difference was not significant ($Z = -0.077$, $p > 0.5$). While honeybees started to attack treatments (after 17 ± 39 seconds) significantly earlier than controls (71 ± 66 seconds; $Z = -2.468$, $p < 0.02$), bumblebees did not attack the controls. The difference in time to attack treatments between honeybees and bumblebees (61 ± 81 seconds) was not significant ($Z = -1.093$, $p > 0.2$).

Discussion

Our data clearly show that small hive beetles do invade bumblebee colonies and readily oviposit there. Contrary to previous findings, however, bumblebees are not helpless but show defensive behaviours. Nevertheless, there are quantitative (worker force) and qualitative differences (hygienic behaviour) between *A. mellifera* and *B. impatiens*.

Our data confirm that small hive beetles prefer bee colonies to empty hive boxes (Spiewok and Neumann, 2006a), the latter providing only hiding places from daylight (Schmolke, 1974), but not emitting odour cues used by the beetles for orientation (Elzen et al., 1999; Torto et al., 2005; Spiewok and Neumann, 2006a). Furthermore, the data suggest that bumblebee colonies are equally or even more attractive to small hive beetles as honeybee colonies of similar size (Fig. 2). Although adult honeybees in combination with hive products are highly attractive for free-flying small hive beetles (Elzen et al., 1999, 2000; Suazo et al., 2003), the number of bees in a colony or colony weight did not correlate with the number of small hive beetles found in that colony. So, stronger colonies were not necessarily more attractive, which may be attributed to the rather small differences in colony strength in our experiment. In the light of similar results from honeybee field colonies (Spiewok and Neumann, 2006b; Neumann and Hoffmann, 2007), this suggests that colony phenotypes are unlikely to trigger small hive beetle host finding.

Our finding of small hive beetle eggs in the debris of one honeybee colony confirms cryptic low-level reproduction (Spiewok and Neumann, 2006c) as an alternative to the usual highly destructive mass reproduction (Lundie, 1940). In all bumblebee colonies beetles had not only invaded, seeking shelter or food, but had also started to lay eggs. This indicates that the bumblebee colonies had been accepted as a suitable breeding ground, thus serving as an alternative host for the small hive beetle. Prior to release, all beetles were kept only on a honey and water diet, so that the females were not ready to oviposit immediately, but had to feed on a protein source first. Hence, protein foraging by small hive beetles is possible in bumblebee nests, supporting earlier findings on successful life cycle completion in association with bumblebee colonies (Ambrose et al., 2000). Considering that within a time window of 36 hours, the beetle females had to leave the release box, find and enter a host colony, locate the food stores and feed on protein diet (e.g. pollen), mate, and oviposit, the host finding process likely did not include a prolonged period of searching. This not only confirms that bumblebee colonies serve as alternate hosts when infested experimentally in the laboratory (Ambrose et al., 2000; Stanghellini et al., 2000), but moreover indicates that small hive beetles, when searching for a host colony, are able to locate and may readily choose bumblebee nests instead of honeybee hives, thereby confirming recent findings of natural infestations

of commercial bumblebee colonies in the field (Spiewok and Neumann, 2006a). However, it has yet to be shown whether small hive beetles actually infest natural nests of *Bombus* species in the field. Clearly, there are differences between commercial bumblebee colonies (Spiewok and Neumann, 2006a) and our experimental boxes compared to natural nests, which are typically underground, preferably in abandoned rodent burrows (Michener, 2000). Nevertheless, olfactory orientation cues may also enable small hive beetle host finding of natural nests, analogous to *Antherophagus* sp. (Coleoptera: Cryptophagidae), which is probably a scavenger of *B. atratus* nests (Gonzalez et al., 2004). Moreover, naturally occurring honeybee (*A. m. scutellata*) nests can also be found underground having only small entrance tubes, and may nevertheless be infested with small hive beetles (PN, pers. obs.). Finally, many small hive beetles were found in feral honeybee colonies in Australia (Somerville, 2003), which can also have very small entrances. In conclusion, our data suggest that bumblebee colonies, be they commercial or wild, are likely to get infested by small hive beetles and if so, can serve as alternate hosts.

The removal rates of eggs demonstrate that, like honeybees (Neumann and Härtel, 2004), bumblebees are able to efficiently remove small hive beetle eggs. They are, however, considerably less efficient if the eggs are hidden in cracks. While in honeybees it appears that tongue length may be the limiting factor in the removal of protected eggs (Neumann et al., 2003), *B. impatiens* workers tend to remove such eggs less proficiently even though in this species tongue length does not differ substantially from honeybees (Harder, 1985; Durka, 2002). Surprisingly, bumblebees attacked the small hive beetle eggs more often than honeybees, and removed the eggs much faster, thereby confirming our finding that bumblebees are very efficient egg removers given that the eggs are exposed and easily accessible. Correspondingly, bumblebees ignored the controls more often than the eggs. Honeybees, on the other hand, investigated both eggs and controls more often than bumblebees. While honeybees usually keep high hygienic standards within the hive and are thus generally intolerant of foreign objects (Seeley, 1985), bumblebees tend to ignore inanimate items (see below). Since eggs are usually eaten, however, egg removal is instantly rewarding to the individual in the form of nutrient gain. Still, although bumblebee colonies may remove a large proportion of the small hive beetle eggs present in the nest, there will likely remain a fair number of protected eggs which will eventually hatch into larvae.

The removal rates of larvae were also comparable to the performance observed in honeybees (Neumann and Härtel, 2004; Spiewok and Neumann, 2006b). Most colonies removed all larvae within 24 hours, and temporary differences in removal rates between field and laboratory colonies did not persist. Bumblebees thus quickly respond to the presence of small hive beetle larvae and are able to dispose of them, thereby preventing

severe damage of the nest. Honeybee workers removed slightly more larvae than bumblebees, but also removed some of the controls. Again, general differences in hygienic behaviour (Seeley, 1985; Goulson, 2003b) likely account for this. The high number of investigations of controls in honeybees was probably due to the longer retention period in the runway, as opposed to the larvae being removed quickly. Honeybees detected the presence of larvae earlier (investigate), but did not attack earlier than bumblebees. Thus, despite some differences in the sequence of actions, bumblebees are equally responsive to small hive beetle life stages as honeybees. The quantitative differences in our data are probably attributable to the great differences in colony strength between the two bee species (Michener, 1974). Average colony size differs between commercial honeybee and *Bombus* colonies by two orders of magnitude, i.e. bumblebee colonies are considerably smaller (Michener, 1974, 2000). This means that fewer workers can engage in colony defence (Michener, 1974), especially if the colony is additionally challenged and many workers are busy with other tasks (e.g. thermoregulation as in our field colonies, see differences in removal rates of larvae, Fig. 5). Our analysis of the colony phenotype data points in the same direction in that the stronger colonies tend to remove small hive beetle larvae faster. Furthermore, due to their different nesting biology, bumblebee hygienic behaviour may be different compared to honeybees. Most bumblebee species nest in the ground (see above) and use nesting material such as grass, moss, hair or wool (Michener, 2000). Hence they tolerate foreign objects to a greater extent than honeybees. Indeed, dead adult beetles can even be included in the involucre (Gonzalez et al., 2004) and remained in some of our test colonies after 24 hours (data not shown).

Although we used the same bumblebee species derived from Koppert Biological Systems and also housed some of our colonies in the laboratory, our findings differ substantially from those made by Stanghellini et al. (2000), who did not observe any colony defence whatsoever. This may be due to differences in colony size (100–200 bees in Stanghellini et al.'s as opposed to 70–400 bees in our experiment) or overall colony health (nest parasite load was not quantified in either study). While we allowed the bees to fly out to the field, however, Stanghellini et al. confined them to the laboratory, so in their study the bees may have not been able to show the whole range of natural behaviours.

Since small hive beetles do not complete their entire life cycle within the beehive, but pupate in the soil (Lundie, 1940), the adults have to find a host colony after eclosion. The small hive beetle as an active flyer can cover distances of several kilometres (c.f. Neumann and Elzen, 2004), and can easily find even cryptic wild honeybee nests following odour cues (see above). However, where host population size is small, i.e. density of host colonies is low, a more opportunistic approach may increase the chances of finding a suitable host within a reasonably

short time (thereby reducing the risks of predation, desiccation, starvation, etc.). In light of the adaptive value of lower host specificity, it is not surprising that several other macroparasites of social insects may also switch hosts within or even across genera. Indeed, *Epuraea depressa* (Coleoptera: Nitidulidae) is reported from the nests of different bumblebee species (Scott, 1920; Cumber, 1949). Likewise, the greater wax moth *Galleria mellonella*, which is normally associated with colonies of different honeybee species (Williams, 1997), was also found in nests of meliponids (Nogueira-Neto, 1953) and bumblebees (Oertel, 1963; Spiewok and Neumann, 2006a). Moreover, the lesser wax moth *Achroia grisella*, which usually infests honeybee colonies (Williams, 1997), has also been reported from stingless bees (Cepeda-Aponte et al., 2002). A further example is the bumblebee wax moth *Aphomia sociella*, which infests various *Bombus* species (Free and Butler, 1959; Pouvreau, 1967) but is also rarely found in honeybee colonies (Toumanoff, 1939). Finally, small hive beetles have also been reported to naturally infest colonies of stingless bees (*Dactylurina staudingerii*) in West Africa (Mutsaers, 2006). Considering that small hive beetles do switch to non-*Apis* hosts within their endemic range, it is not surprising that in their new ranges other social bees may serve as alternate hosts. In light of these earlier reports and of our data it appears that small hive beetles are less host specific than previously thought. In fact, several transmission events of parasites from invaders to native species are already known (Prenter et al., 2004). The taxonomic proximity and ecological similarities within the family Apidae (Michener, 1974, 2000) seem to generally facilitate host switches of macroparasites.

Aside from this, there is another reason to expect low host specificity in the small hive beetle. Originating from a family of opportunistic scavengers (the sap beetles, Nitidulidae) with a low level of specialization (Morse, 1998), small hive beetles seem to be pre-adapted for host switch. They are able to sustain themselves and reproduce not only on hive products of both intact and absconded honeybee colonies, in stingless bee and commercial bumblebee colonies (see above), but also on a variety of fruits (Ellis et al., 2002), as well as on the sparse resources contained in the debris of functioning honeybee colonies (Spiewok and Neumann, 2006c). Thus, the small hive beetle can be expected to infest still other social bee species than the ones it has been found in so far.

In conclusion, our findings show that small hive beetles do not prefer honeybee hives over bumblebee colonies, so native pollinators may serve as alternate hosts. Field surveys are therefore necessary to evaluate the actual infestation status of wild bumblebee colonies and its impact on the conservation status of bumblebee species. But even though bumblebees lack a co-evolutionary history with the small hive beetle, they are able to defend their colonies against this nest intruder considerably well. Thus, the observed defence mechanisms must be part of a generalistic defence system suitable for

defence against multiple attackers (Michener, 1974; Thompson 1994). This supports general patterns of host-parasite co-evolution in that specialization is harder to accomplish for the victims, but it is possible for them to maintain more general defences towards multiple attackers (Thompson, 1994; see also Jokela et al., 2000).

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